

AD _____

Award Number: DAMD17-03-1-0411

TITLE: Phasic Dopaminergic Signaling and the Presymptomatic Phase of Parkinson's Disease

PRINCIPAL INVESTIGATOR: Paul A. Garris, Ph.D.
Tim Schallert
Byron A. Heidenreich

CONTRACTING ORGANIZATION: Illinois State University
Normal, Illinois 61790-3040

REPORT DATE: July 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20050302 180

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)			2. REPORT DATE July 2004	3. REPORT TYPE AND DATES COVERED Annual (1 Jul 2003 - 30 Jun 2004)
4. TITLE AND SUBTITLE Phasic Dopaminergic Signaling and the Presymptom Phase of Parkinson's Disease			5. FUNDING NUMBERS DAMD17-03-1-0411	
6. AUTHOR(S) Paul A. Garris, Ph.D., Tim Schallert Byron A. Heidenreich				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Illinois State University Normal, Illinois 61790-3040 E-Mail: pagarri@ilstu.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) The purpose of this project is to investigate the relationship between phasic dopaminergic signaling and behavior in an animal model of Parkinson's disease. The overall hypothesis is that, in rats with partial dopamine lesions mimicking the preclinical phase of Parkinson's disease, deficits in phasic dopaminergic signaling are associated with behavioral deficits. Phasic dopaminergic signaling will be characterized by chemical microsensors measuring dopamine, and electrophysiology is used to monitor the effect of dopamine on target cells. Several behavioral tests will be developed and assessed to identify deficits that occur during partial dopamine depletion. The major finding for Year 1 is that the amplitude of dopamine transients, electrically evoked by stimulation parameters consistent with phasic signaling, decreases in proportion to the degree of dopamine lesion, supporting the hypothesis of a deficit in phasic dopaminergic signaling following partial dopamine denervation. Five behavioral tests have been assessed but to date, only forelimb use asymmetry shows a similar dependence on the degree of dopamine lesion as seen with phasic signaling. Instrumentation and other technical work are ongoing in preparation for subsequent work in Years 2 through 4 of the project.				
14. SUBJECT TERMS Parkinson's disease, dopamine, compensation, phasic signaling, microsensors, electrophysiology, behavior			15. NUMBER OF PAGES 12	16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4-7
Key Research Accomplishments.....	7-8
Reportable Outcomes.....	8
Conclusions.....	8
References.....	8-9
Appendices.....	10-12

Introduction

The long-term objective of this project is to better understand compensatory adaptation in Parkinson's disease. To this end, an important mode of dopaminergic neurotransmission, phasic signaling, will be examined in an animal model. It is well established that classic parkinsonian symptoms are associated with the loss of nigrostriatal dopaminergic neurons. However, symptoms do not appear until severe denervation due to the compensatory normalization of dopaminergic tone. Little is known about the relationship between denervation and phasic signaling, which generates concentration spikes on top of ambient dopamine levels. Evidence obtained by us indicates that partial lesions decrease spike concentration without altering tone. We propose that degraded phasic responses mediate subtle behavioral deficits observed in these animals and preclinically in humans. Phasic signaling will be characterized using state-of-the-art microsensor and behavioral approaches. Overall, this project will advance knowledge of basic neurodegenerative processes, potentially leading to earlier and improved diagnosis and treatment.

Body

Statement of Work for Year 1 (verbatim from proposal)

Phasic dopaminergic signaling will be characterized in anesthetized animals using real-time chemical microsensors.

Two asymmetry tests assessing sensorimotor function will be performed on each of the animals above.

The technique of quasi-simultaneous voltammetry and electrophysiology will be established at Illinois State University.

Quasi-simultaneous voltammetry and electrophysiology will be used to assess adaptation of striatal target cells to phasic dopaminergic signaling in anesthetized animals.

Phasic dopaminergic signaling will be characterized in anesthetized animals using real-time chemical microsensors

One key basis for the proposal was our previous demonstration of a potential deficit in phasic dopaminergic signaling, a transient increase or spike in extracellular dopamine, in rats with partial denervation of nigrostriatal dopaminergic neurons (Bergstrom and Garris, 2003). This finding is significant because of emerging importance attributed to phasic dopaminergic signaling in behavior (Schultz, 2000; Garris and Rebec, 2002; Wightman and Robinson, 2002) and because of potential relevance to the preclinical phase of Parkinson's disease. However, Bergstrom and Garris (2003) was criticized for using supraphysiological parameters for electrical stimulation. Consequently, the study proposed in the Statement of Work for Year 1 used more physiological parameters to investigate the relationship between dopamine depletion and electrically evoked dopamine transients. This study is now complete, and a manuscript is in preparation. Results have also been described in an abstract submitted to the 2004 Annual Meeting for the Society of Neuroscience (Sandberg et al.).

In the new study, we demonstrated that stimulation parameters mimicking tonic dopamine signaling (5 Hz), which is thought to maintain an ambient, low concentration of extracellular dopamine, elicited responses that were insensitive to dopamine lesions; however, the amplitude of dopamine transients, electrically evoked by stimulation parameters mimicking phasic dopamine signaling (4 pulses at 20 Hz), decreased in proportion to the loss of striatal dopaminergic terminals (Figure 1A and B, respectively). Taken together, these results are in excellent agreement with Bergstrom and Garris (2003) and support our contention that compensatory adaptation following partial dopamine lesions that mimick the preclinical phase of Parkinson's disease maintains tonic but not phasic dopaminergic signaling.

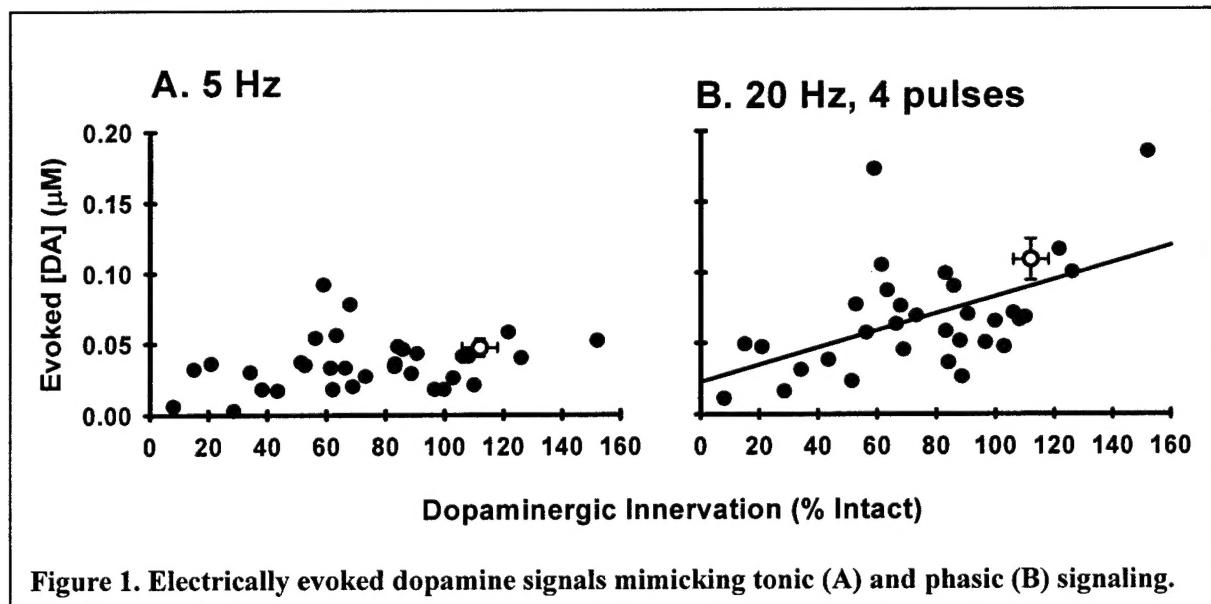


Figure 1. Electrically evoked dopamine signals mimicking tonic (A) and phasic (B) signaling.

These experimental results supported by USAMRMC 03281055 will be combined with theoretical work supported by NIH NS 35298-02 (PI: Garris) for a manuscript detailing compensatory adaptation during the preclinical phase of Parkinson's disease (Sandberg et al., in preparation). Theoretical work for the combined study utilized a mathematical model, describing the regulation of brain extracellular dopamine by release, uptake and diffusion (Venton et al., 2003), that was modified by us to simulate the effects of dopamine lesions (Garris et al., in preparation). Simulations closely mirrored experimental measurements of electrically evoked dopamine levels, suggesting that the new model accurately describes dopaminergic neurotransmission in the lesioned rat. The model is further used to predict effects of L-DOPA, a drug used as the primary treatment for Parkinson's disease, on tonic and phasic dopaminergic signaling. Because of its potential impact on the field, we anticipate submitting Sandberg et al. (in preparation) to a high profile journal such as *PNAS*.

Two asymmetry tests assessing sensorimotor function will be performed on each of the animals above

The rationale for this work assessing sensorimotor function is straightforward: in partially dopamine-denervated rats, deficits in phasic dopaminergic signaling should produce behavioral deficits. The longer term objective is to measure phasic dopamine signals in lesioned animals during behavior (Years 3 and 4). The first step is to identify behaviors with deficits that emerge

following partial lesions (Years 1 and 2). The original Statement of Work for Year 1 was to assess two tests, forelimb use asymmetry and vibrissae-evoked forelimb placement, in lesioned animals and subsequently perform voltammetric measurements of dopamine under anesthesia. This plan has been amended and expanded to assess several behaviors initially without performing voltammetry. The rationale is that first surveying several behavioral tests, which can be performed fairly quickly, will be more efficient before using voltammetry, a slower throughput technique..

In addition to the forelimb use asymmetry and vibrissae-evoked forelimb placement, three other tests have been assessed to date: passive initiation threshold - degree of weight shift needed to initiate an adjusting step to regain center of gravity; engagement - time to orient the head in response to a whisker stroke; and disengagement - time to orient the head to a whisker stroke while engaged in eating. The three additional tests were recommended by Co-PI Schallert. The primary conclusion is that, except for forelimb use asymmetry showing deficits at partial lesions (Fig. 2), all other tests require a severe lesion before deficits emerge (e.g., see Fig. 3 for vibrissae-evoked forelimb placement). The results of this study have been described in an abstract submitted to the 2004 Annual Meeting for the Society of Neuroscience (Mithyantha et al.). In addition to what is already described in the Statement of Work for Year 2 and following recommendations from Co-PI Schallert, plans are being made to assess another test, reaction time (Smith et al., 2002), in the coming year, before combining behavior and voltammetry measurements. Ideally, we would like to identify more than one test that shows deficits following partial lesions and then, in Years 3 and 4, select the test that would be most convenient to monitor dopamine simultaneously with behavior.

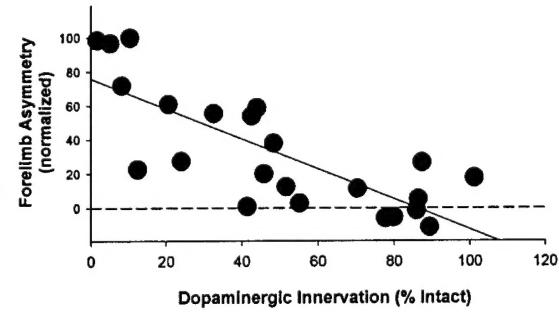


Figure 2. Forelimb asymmetry.

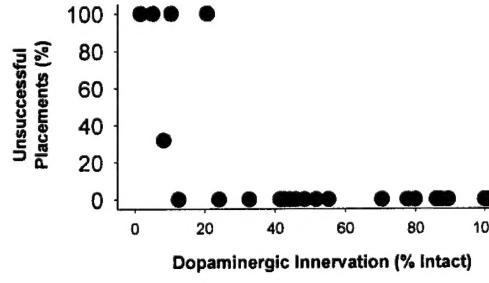


Figure 3. Vibrissae-evoked placement.

An unexpected finding, observed during the passive initiation threshold test, is also being explored in conjunction with a study supported by NIH NS19608 and NS23979 (Co-PI Schallert). The finding is that, while the adjusting step length is increased for the forelimb contralateral to the lesion, indicative of a deficit, it is decreased for the forelimb ipsilateral to the lesion, suggesting compensation by the intact striatum. The results of this study have been described in an abstract submitted to the 2004 Annual Meeting for the Society of Neuroscience (Woodlee et al.). Whether phasic dopaminergic signaling has a role in this novel compensation is not known, but could be explored in this project time permitting or in a future project.

The technique of quasi-simultaneous voltammetry and electrophysiology will be established at Illinois State University

We have been working with Dr. Colin McKinney, Ph.D., director of the electronic shop for the Department of Chemistry at the University of North Carolina, to develop a system for quasi-simultaneous voltammetry and electrophysiology. Originally in the proposal, the system would consist of a combination of commercial instruments and instruments custom built and designed by McKinney. Instead, we have opted to have McKinney design and construct the entire system. Although taking a little longer, the end result will be a better and more flexible, state-of-the-art system for a similar price.

In the meantime, recording striatal single units has been established at ISU using a commercial electrophysiology system in the laboratory of Co-PI Heidenreich. Figure 4A and B shows units recorded with a conventional, saline-filled glass capillary and carbon-fiber microelectrode, respectively. Establishing the latter is important, because carbon-fiber microelectrodes will be used for quasi-simultaneous voltammetry and electrophysiology. Current work is directed at integrating iontophoresis into the electrophysiological measurements. Ideally, a multibarreled

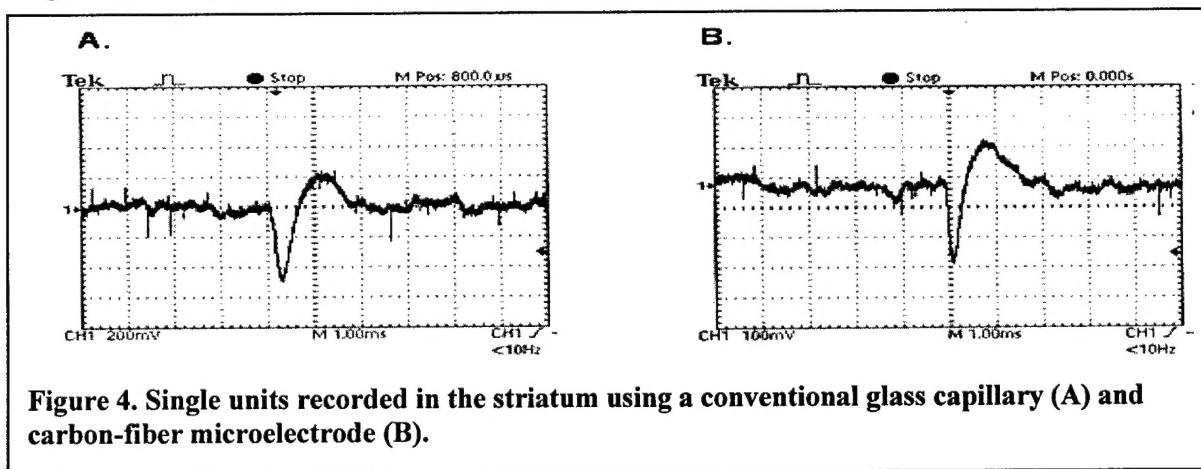


Figure 4. Single units recorded in the striatum using a conventional glass capillary (A) and carbon-fiber microelectrode (B).

pipette, with the middle barrel containing a carbon fiber for recording, will be used. However, if electrical interference occurs, the carbon-fiber recording electrode and iontophoresis pipette may need to be fabricated separately, glued together, and used as a “piggy-back” assembly.

Quasi-simultaneous voltammetry and electrophysiology will be used to assess adaptation of striatal target cells to phasic dopaminergic signaling in anesthetized animals

These measurements, which were originally scheduled to start in Year 1 and continue during the entire Year 2, will commence shortly, once construction of the custom-made instrumentation is completed, and set-up and testing has occurred.

Key Research Accomplishments

- Characterized sensitivity of dopamine transients, electrically evoked by physiological parameters, to partial dopamine lesions
- Developed mathematical basis for maintenance of tonic but not phasic dopaminergic signaling across the preclinical lesion range in Parkinson's disease
- Predicted effects of L-DOPA on phasic dopamine signaling in Parkinson's disease
- Characterized sensitivity of five sensorimotor tests to partial dopamine lesions

- Identified novel compensation in intact striatum of unilaterally dopamine-lesioned rat (in progress)
- Designed and constructed custom-made system for quasi-simultaneous voltammetry and electrophysiology (in progress)
- Established recording of striatal single units using a carbon-fiber microelectrode at ISU

Reportable Outcomes

Sandberg SG, Mithyantha J, Pakdeeronachit S, Leesman CW and Garris PA. 2004. Further support for 'Passive Stabilization' of striatal dopamine in an animal model of preclinical Parkinson's disease. Abstract submitted to the Annual Meeting for the Society for Neuroscience (San Diego, CA). Appendix I.

Mithyantha J, Pakdeeronachit S, Woodlee MT, Schallert T and Garris PA. 2004. Partial unilateral nigrostriatal dopamine denervation in the rat: behavioral correlates potentially associated with reduced phasic dopamine signaling. Abstract submitted to the Annual Meeting for the Society for Neuroscience (San Diego, CA). Appendix II.

Woodlee MT, Mithyantha J, Kane JR, Chang J, Garris PA and Schallert T. 2004. Functional reorganization of the intact hemisphere following unilateral nigrostriatal dopamine depletion: implications for Parkinsonian models. Abstract submitted to the Annual Meeting for the Society for Neuroscience (San Diego, CA). Appendix III.

Conclusions

The primary result of work during Year 1 was the best support to date for a novel hypothesis describing dopaminergic neurotransmission during Parkinson's disease. The hypothesis states that tonic, but not phasic, dopaminergic signaling is maintained during the preclinical phase. A neurochemical mechanism for the hypothesis is also provided, and the hypothesis is based on both experimental and theoretical evidence. The loss of phasic dopaminergic signaling should be associated with behavioral deficits. If identified, these deficits could be used as a basis for early diagnosis of Parkinson's disease, spurring early treatment. Moreover, the theoretical model could be used for predicting drug effects on extracellular dopamine levels in Parkinson's disease, and therefore drive new treatment and understand limitations of current treatment.

The other work accomplished during Year 1 was directed at setting the stage for subsequent experiments in Years 2 through 4. With the addition of three new tests, behavioral assessment has been expanded. Establishment of quasi-simultaneous voltammetry and electrophysiology is slightly behind schedule, but a better instrument will be developed, benefiting this and other projects in the long run.

References

Bergstrom BP, Garris PA (2003) 'Passive stabilization' of striatal extracellular dopamine across the lesion spectrum encompassing the presymptomatic phase of Parkinson's disease: a voltammetric study in the 6-OHDA-lesioned rat. *J Neurochem* 87: 1224-1236.

Garris PA, Rebec GV (2002) Modeling fast dopamine neurotransmission in the nucleus accumbens during behavior. *Behav Brain Res* 137: 47-63.

Schultz W (2000) Multiple reward signals in the brain. *Nat Rev Neurosci* 1: 199-207.

Smith AD, Amalric M, Koob GF, Zigmond MJ (2002) Effect of bilateral 6-hydroxydopamine lesions of the medial forebrain bundle on reaction time. *Neuropsychopharmacology* 26: 756-764.

Venton BJ, Zhang H, Garris PA, Phillips PE, Sulzer D, Wightman RM (2003) Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. *J Neurochem* 87: 1284-1295.

Wightman RM, Robinson DL (2002) Transient changes in mesolimbic dopamine and their association with 'reward'. *J Neurochem* 82: 721-735.

Appendices

Appendix I: Sandberg et al. (2004) Abstract submitted to Society for Neuroscience Meeting

Contact Info Stefan G. Sandberg
Illinois State Univ
Normal
IL 61790
USA
Phone: 309-438-3083
ssandbe@ilstu.edu

Date/Time Last Modified: 05/19/2004 at 1:16 pm GMT

Presentation Type: Poster Only

Theme 1: Synaptic Transmission and Excitability

Subtheme 1: Neurotransmitters

Topic 1: Catecholamines

Theme 2: Neurological and Psychiatric Conditions

Subtheme 2: Neurodegenerative Disorders

Topic 2: Parkinson's Disease: Models

Abstract Title: FURTHER SUPPORT FOR "PASSIVE STABILIZATION" OF STRIATAL DOPAMINE IN AN ANIMAL MODEL OF PRECLINICAL PARKINSON'S DISEASE

Contributing Authors: S.G. Sandberg*; J. Mithyantha; S. Pakdeeronachit; C.W. Leesman; P.A. Garris

Institutions: Cell. Integr. Physiol. Sect., Dept. Biol. Sci., Illinois State Univ, Normal, IL, USA

Key words:

Abstract: We previously proposed that steady-state concentrations of extracellular dopamine (DA) in the striatum are normalized across the denervation range mimicking preclinical Parkinson's disease (0-80%) without up-regulated DA release or down-regulated DA uptake (2003 J Neurochem 87:1224). A consequence of this proposed compensatory model called "passive stabilization" is that the amplitude of DA concentration spikes or transients would not be normalized. Here we further investigated passive stabilization utilizing physiological stimulation parameters: 5 Hz, 2 s trains to evoke steady-state DA levels and 20 Hz, 4 p trains to evoke DA spikes. DA was monitored by FSCV in anesthetized rats with partial, unilateral 6-OHDA lesions. The stimulating electrode was placed in the MFB. Voltammetric traces were mathematically analyzed to obtain DA release and uptake parameters. Steady-state DA levels remained unchanged, whereas amplitudes of DA transients decreased in proportion to denervation. In addition, both DA release and uptake parameters decreased proportionally with denervation. These results are consistent with our previous study using supra-physiological stimulation and the passive stabilization hypothesis. They may also have implications for the relationship between the two modes of DA signaling and denervation. DA neurons fire at 5 Hz on average during tonic signaling, producing an ambient or steady-state DA level. During phasic signaling, in contrast, burst firing at around 20 Hz generates a transient spike of DA. Thus, the maintenance of evoked steady-state DA levels, but not evoked DA spikes, may indicate that the partially denervated striatum has the capacity to normalize tonic but not phasic DA signaling. Furthermore, this apparent deficit in phasic DA signaling may have associated behavioral deficits.

Support Contributed By: NIH NS 35398-02 and USAMRMC 03281055.

Appendix II: Mithyantha et al. (2004) Abstract submitted to Society for Neuroscience Meeting

Contact Info Paul A Garris
Illinois State Univ
Normal
IL 61790-4120
USA
Phone: 309-438-2664
Fax: 309-438-3722
pagarri@ilstu.edu

Date/Time Last Modified: 05/19/2004 at 5:47 pm GMT

Presentation Type: Poster Only

Theme 1: Neurological and Psychiatric Conditions

Subtheme 1: Neurodegenerative Disorders

Topic 1: Parkinson's Disease: Models

Theme 2: Motor Systems

Subtheme 2: Basal Ganglia

Topic 2: Behavior

Abstract Title: PARTIAL UNILATERAL NIGROSTRIATAL DOPAMINE DENERVATION IN THE RAT: BEHAVIORAL CORRELATES POTENTIALLY ASSOCIATED WITH REDUCED PHASIC DOPAMINE SIGNALING

Contributing Authors: J. Mithyantha¹; S. Pakdeeronachit¹; M.T. Woodlee²; T. Schallert^{2,3}; P.A. Garris^{1*}

Institutions: 1. Dept Biolog Sci, Illinois State Univ, Normal, IL, USA 2. Inst Neurosci, Univ Texas, Austin, TX, USA 3. Dept Neurosurgery, Univ Michigan, Ann Arbor, MI, USA

Key words: PARKINSON, STRIATUM, BEHAVIOR, DOPAMINE

Abstract: Cardinal symptoms of Parkinson 's disease do not present until about 75% of striatal dopamine content is lost. However, emerging evidence suggests that subtle sensory, motor, learning or emotional changes, especially during stress, may occur during the preclinical phase. We have recently identified a deficit in phasic dopamine signaling in the rat striatum following partial 6-hydroxydopamine induced dopamine depletion (2003 J Neurochem 87:1224). In particular, the amplitude of electrically evoked dopamine concentration spikes decreased in proportion to dopamine levels across the preclinical denervation range. The behavioral consequence of this neurochemical deficit is not established, but phasic dopamine signaling is normally activated by salient stimuli and related to reward error prediction, behavioral switching and associative learning. It is therefore tempting to speculate that reduced subsecond dopamine signaling may be related to one or more preclinical symptoms in Parkinson 's disease. A goal of the present study was to identify behavioral measures that are linearly related to striatal dopamine denervation. The following tests were evaluated: forelimb-use asymmetry; vibrissae-evoked forelimb placing; orienting movement time - time to orient the head in response to a whisker stroke; disengagement - time to orient while engaged in eating; and passive initiation threshold - degree of weight shift needed to initiate an adjusting step to regain center of gravity. After identifying the appropriate test, the next step is monitoring phasic dopamine concentration spikes during the behavior.

Support Contributed By: USAMRMC o3281055 and NS19608

Appendix III: Woodlee et al. (2004) Abstract submitted to Society for Neuroscience Meeting

Presentation Poster Only

Type:

Theme 1: Neurological and Psychiatric Conditions

Subtheme 1: Neurodegenerative Disorders

Topic 1: Parkinson's Disease: Models

Theme 2: Motor Systems

Subtheme 2: Basal Ganglia

Topic 2: Behavior

Abstract Title: **FUNCTIONAL REORGANIZATION OF THE INTACT HEMISPHERE FOLLOWING UNILATERAL NIGROSTRIATAL DOPAMINE DEPLETION: IMPLICATIONS FOR PARKINSONIAN MODELS**

Contributing Authors: M.T. Woodlee¹; J. Mithyantha³; J.R. Kane²; J. Chang²; P.A. Garris³; T. Schallert^{1,2,4}

Institutions: 1. Inst Neurosci, 2. Dept Psych, Univ Texas, Austin, TX, USA 3. Dept Biol Sci, Illinois State Univ, Normal, IL, USA 4. Dept Neurosurgery, Univ Michigan, Ann Arbor, MI, USA

Key words: DOPAMINE, PLASTICITY, PARKINSON, BEHAVIOR

Abstract: Rats sustaining unilateral 6-hydroxydopamine-induced striatal dopamine depletion show parkinsonian symptoms in the opposite forelimb. Here we describe a new behavioral test involving "catch-up" steps in response to experimenter-imposed shifts of the rat's center of gravity. In this test the experimenter restrains all but one forelimb from resting on a flat surface and then slowly moves the rat's body forward over the planted limb. The amount of forward movement needed to trigger a catch-up step in the planted limb (to regain center of gravity) is recorded for each forelimb. We found that a greater shift is required to elicit a catch-up step in the parkinsonian forelimb relative to normal animals. Surprisingly, the unimpaired forelimb of lesioned animals requires a smaller weight shift to elicit such a step than does either limb of unlesioned controls, suggesting a reorganization of circuitry in the intact hemisphere. Data presented will include (1) the time course of the effect's development, (2) its relation to levels of dopamine depletion and striatal c-fos expression, and (3) the effect of drugs such as apomorphine and haloperidol. The neural and behavioral adaptations observed may depend on a combination of degenerative events and new motor experience, and may allow for improved overall function. We conclude that components of the dopamine signaling system are upregulated in the intact striatum of unilaterally 6-OHDA-lesioned rats relative to unlesioned controls. This emphasizes the importance of using appropriate controls in experiments involving hemiparkinsonian rats, and furthers the notion that compensatory plasticity plays an important role in the brain's response to injury.

Support Contributed By: NIH (NS19608, NS23979) and USAMRMC 03281055